

AMENDMENTS TO THE CLAIMS:

The listing of claims will replace all prior versions, and listings of claims in the application:

LISTING OF THE CLAIMS

1. (Currently Amended) A composition $[[,]]$ comprising a crystal of isolated *Streptococcus pneumoniae* acyl carrier protein synthase.

2. (Original) The composition of claim 1, wherein said crystal effectively diffracts X-rays, and permits the determination of the atomic coordinates of said acyl carrier protein synthase to a resolution of about 2.0 Å.

3. (Currently Amended) The composition of claim 1, wherein said *Streptococcus pneumoniae* acyl carrier protein synthase has an active site cavity that binds 3',5'-adenosine diphosphate. ~~comprising the 3',5'-adenosine diphosphate binding site shown in Figure 9.~~

4. (Original) The composition of claim 1, wherein said *Streptococcus pneumoniae* acyl carrier protein synthase is a homotrimer, wherein each protomer comprises the following structural motifs:

- (a) a three-stranded anti-parallel β -sheet formed by strands $\beta 1$, $\beta 5$, and $\beta 4$;
- (b) a long α -helix that packs diagonally against said β -sheet, together with α -helices $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$ of an anti-parallel four helical bundle; and
- (c) a long, extended loop with a two-strand anti-parallel β -sheet comprising strands $\beta 2$ and $\beta 3$,

wherein said structural motifs (a), (b), and (c) are organized such that said long helix $\alpha 4$ runs through said homotrimer, and is surrounded by the remainder of said structural motifs, as shown in Figures 8(B) and 8(C).

5. (Original) The composition of claim 1, wherein said crystal belongs to orthorhombic space group $P2_12_12_1$, with unit cell dimensions of $a = 49.8 \text{ \AA}$, $b = 59.6 \text{ \AA}$, and $c = 114.7 \text{ \AA}$, or monoclinic space group $C2$, with unit cell dimensions of $a = 120.2 \text{ \AA}$, $b = 62.3 \text{ \AA}$, $c = 51.7 \text{ \AA}$, and $\beta = 98.7^\circ$.

6. (Original) The composition of claim 1, wherein said acyl carrier protein synthase comprises selenocysteine or selenomethionine.

7. (Original) The composition of claim 1, wherein said acyl carrier protein synthase comprises a heavy metal atom.

8. (Original) The composition of claim 1, further comprising a chemical compound complexed covalently or non-covalently with said crystal.

9. (Original) The composition of claim 8, wherein said chemical compound is 3',5'-adenosine diphosphate.

10. (Currently Amended) The composition of claim 9, wherein said crystal belongs to monoclinic space group $C2$, with unit cell dimensions of $a = 120.2 \text{ \AA}$, $b = 62.3 \text{ \AA}$, $c = 51.7 \text{ \AA}$, and $\beta = 98.7^\circ$.

11. (Original) The composition of claim 1, wherein said *Streptococcus pneumoniae* acyl carrier protein synthase has the amino acid sequence shown in SEQ ID NO:1.

12. (Original) The composition of claim 1, wherein said crystal has the atomic coordinates shown in Table 3 or Table 4.

13. (Original) The composition of claim 9, wherein said crystal has the atomic coordinates shown in Table 5.

14. (Currently Amended) A composition $[[,]]$ comprising a crystal of *Streptococcus pneumoniae* acyl carrier protein synthase having the amino acid sequence shown in SEQ ID NO: 1 wherein methionine is substituted with selenomethionine,

wherein said acyl carrier protein synthase is a homotrimer, wherein each protomer comprises the following structural motifs:

- (a) a three-stranded anti-parallel β -sheet formed by strands $\beta 1$, $\beta 5$, and $\beta 4$;
- (b) a long α -helix that packs diagonally against said β -sheet, together with α -helices $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$ of an anti-parallel four helical bundle; and
- (c) a long, extended loop with a two-strand anti-parallel β -sheet comprising strands $\beta 2$ and $\beta 3$,

wherein said structural motifs (a), (b), and (c) are organized such that said long helix $\alpha 4$ runs through said homotrimer, and is surrounded by the remainder of said structural motifs, as shown in Figures 8(B) and 8(C);

wherein when said acyl carrier protein synthase is in native form, said crystal belongs to orthorhombic space group $P2_12_12_1$, with unit cell dimensions of $a = 49.8 \text{ \AA}$, $b = 59.6 \text{ \AA}$, and $c = 114.7 \text{ \AA}$, or monoclinic space group $C2$, with unit cell dimensions of $a = 120.2 \text{ \AA}$, $b = 62.3 \text{ \AA}$, $c = 51.7 \text{ \AA}$, and $\beta = 98.7^\circ$, and wherein said crystal has the atomic coordinates shown in Table 3 or Table 4, respectively; and

wherein when said acyl carrier protein synthase is complexed with 3'5'-adenosine diphosphate, said crystal belongs to monoclinic space group $C2$, with unit cell dimensions of $a = 120.2 \text{ \AA}$, $b = 62.3 \text{ \AA}$, $c = 51.7 \text{ \AA}$, and $\beta = 98.7^\circ$, and wherein said crystal has the atomic coordinates shown in Table 5.

15. (Withdrawn) An enzyme active site crystal structure comprising the 3'5'-adenosine diphosphate binding site shown in Figure 9.

16. (Withdrawn) The enzyme active site crystal structure of claim 15, wherein said enzyme comprises isolated, properly folded *Streptococcus pneumoniae* acyl carrier protein synthase, or a fragment thereof comprising said active site.

17. (Withdrawn) A method of isolating *Streptococcus pneumoniae* acyl carrier protein synthase, comprising:

- (a) growing said *Streptococcus pneumoniae* in a medium lacking methionine but containing L-selenomethionine;
- (b) preparing a cell extract of said *Streptococcus pneumoniae*;
- (c) centrifuging said cell extract to produce a supernatant fraction, and collecting said supernatant fraction;
- (d) chromatographing said supernatant fraction on a cation exchange column in buffer containing dithiothreitol or β -mercaptoethanol, and collecting fractions containing said *Streptococcus pneumoniae* acyl carrier protein synthase;
- (e) chromatographing said fractions of step (d) on a gel filtration column in buffer containing dithiothreitol or β -mercaptoethanol, and collecting fractions containing said *Streptococcus pneumoniae* acyl carrier protein synthase comprising L-selenomethionine.

18. (Withdrawn) The method of claim 17, further comprising chromatographing said fractions of step (e) on an anion exchange column in buffer containing dithiothreitol or β -mercaptoethanol, and collecting fractions containing said *Streptococcus pneumoniae* acyl carrier protein synthase.

19. (Withdrawn) The method of claim 18, wherein said *Streptococcus pneumoniae* acyl carrier protein synthase has the amino acid sequence shown in SEQ ID NO:1, wherein methionine is replaced with L-selenomethionine.

20. (Currently Amended) ~~Isolated~~ A *Streptococcus pneumoniae* acyl carrier protein synthase isolated by the process of ~~produced by the method of claim 19.~~

- a) growing said *Streptococcus pneumoniae* in a medium lacking methionine but containing L-selenomethionine;
- b) preparing a cell extract of said *Streptococcus pneumoniae*;
- c) centrifuging said cell extract to produce a supernatant fraction, and collecting said supernatant fraction;

- d) chromatographing said supernatant fraction on a cation exchange column in buffer containing dithiothreitol or β -mercaptoethanol, and collecting fractions containing said *Streptococcus pneumoniae* acyl carrier protein synthase;
- e) chromatographing said fractions of step (d) on a gel filtration column in buffer containing dithiothreitol or β -mercaptoethanol, and collecting fractions containing said *Streptococcus pneumoniae* acyl carrier protein synthase comprising L-selenomethionine;
- f) chromatographing said fractions of step (e) on an anion exchange column in buffer containing dithiothreitol or β -mercaptoethanol, and collecting fractions containing said *Streptococcus pneumoniae* acyl carrier protein synthase; and

wherein said *Streptococcus pneumoniae* acyl carrier protein synthase has the amino acid sequence shown in SEQ ID NO:1, wherein methionine is replaced with L-selenomethionine.

21. (Withdrawn) A method of producing a crystal of *Streptococcus pneumoniae* acyl carrier protein synthase that diffracts X-rays, comprising:

- (a) providing *Streptococcus pneumoniae* acyl carrier protein synthase isolated according to claim 19;
- (b) concentrating said acyl carrier protein synthase to 8 mg/ml in a solution containing 10 mM $MgCl_2$, 14 mM KCl, and 20 mM Tris-HCl at pH 7.1 to produce a concentrated protein solution;
- (c) equilibrating a 4 μ l drop of said acyl carrier protein synthase in a solution comprising a mixture of 1:1, v/v, concentrated protein solution as in step (b)/reservoir solution over a 500 μ l reservoir solution comprising 8-15% polyethyleneglycol 4000, 200 mM ammonium sulfate, and 100 mM citrate buffer at pH 4.5; and
- (d) growing a crystal of said acyl carrier protein synthase by vapor diffusion at 294K for at least 4 to 5 days.

22. (Withdrawn) The method of claim 21, further comprising determining a three-dimensional structure of said crystal.

23. (Withdrawn) The method of claim 21, wherein said crystal belongs to orthorhombic space group $P2_12_12_1$, having unit cell parameters $a = 49.8 \text{ \AA}$, $b = 59.6 \text{ \AA}$, $c = 114.7 \text{ \AA}$, or monoclinic space group $C2$, having unit cell parameters $a = 120.2 \text{ \AA}$, $b = 62.3 \text{ \AA}$, $c = 51.7 \text{ \AA}$, $\beta = 98.7^\circ$, comprises one homotrimeric molecule per asymmetric unit, and has the atomic coordinates shown in Table 3 or Table 4.

24. (Withdrawn) The method of claim 21, further comprising testing the ability of a compound to form a complex with an active site of said acyl carrier protein synthase by including said compound in said concentrated protein solution of step (b).

25. (Withdrawn) The method of claim 21, further comprising contacting said crystal of acyl carrier protein synthase and a solution comprising a compound of interest to form a mixture, incubating said mixture to permit said compound to diffuse into said crystal, and determining whether said compound forms a complex with said acyl carrier protein synthase.

26. (Withdrawn) The method of claim 25, wherein said compound is 3',5'-adenosine diphosphate.

27. (Withdrawn) The method of claim 25, wherein when said compound forms a complex with said acyl carrier protein synthase, determining a three-dimensional structure of said acyl carrier protein synthase/compound crystal complex.

28. (Withdrawn) The method of claim 26, wherein said crystal belongs to monoclinic space group $C2$, having unit cell parameters $a = 120.2 \text{ \AA}$, $b = 62.3 \text{ \AA}$, $c = 51.7 \text{ \AA}$, $\beta = 98.7^\circ$, comprises one homotrimeric molecule per asymmetric unit, and has the atomic coordinates shown in Table 5.

29. (Currently Amended) A crystal of *Streptococcus pneumoniae* acyl carrier protein synthase that diffracts X-rays, produced by the method of ~~claim 21~~.

- (a) providing *Streptococcus pneumoniae* acyl carrier protein synthase isolated according to claim 20;
- (b) concentrating said acyl carrier protein synthase to 8 mg/ml in a solution containing 10 mM MgCl₂, 14 mM KCl, and 20 mM Tris-HCl at pH 7.1 to produce a concentrated protein solution;
- (c) equilibrating a 4 µl drop of said concentrated protein solution of step (b) comprising a mixture of 1:1, v/v, acyl carrier protein synthase/reservoir solution in a 500 µl solution comprising 8-15% polyethyleneglycol 4000, 200 mM ammonium sulfate, and 100 mM citrate buffer at pH 4.5; and
- (d) growing a crystal of said acyl carrier protein synthase by vapor diffusion at 294K for at least 4 to 5 days.

30. (Currently Amended) A crystal of *Streptococcus pneumoniae* acyl carrier protein synthase that diffracts X-rays, produced by the method of ~~claim 24~~.

- (a) providing *Streptococcus pneumoniae* acyl carrier protein synthase isolated according to claim 20;
- (b) concentrating said acyl carrier protein synthase to 8 mg/ml in a solution containing a chemical compound of interest, 10 mM MgCl₂, 14 mM KCl, and 20 mM Tris-HCl at pH 7.1 to produce a concentrated protein solution;
- (c) equilibrating a 4 µl drop of said concentrated protein solution of step (b) comprising a mixture of 1:1, v/v, acyl carrier protein synthase/reservoir solution in a 500 µl solution comprising 8-15% polyethyleneglycol 4000, 200 mM ammonium sulfate, and 100 mM citrate buffer at pH 4.5; and
- (d) growing a crystal of said acyl carrier protein synthase by vapor diffusion at 294K for at least 4 to 5 days.

31. (Currently Amended) A crystal of *Streptococcus pneumoniae* acyl carrier protein synthase that diffracts X-rays, produced by the method of ~~claim 25~~.

- (a) providing *Streptococcus pneumoniae* acyl carrier protein synthase isolated according to claim 20;
- (b) concentrating said acyl carrier protein synthase to 8 mg/ml in a solution containing 10 mM MgCl₂, 14 mM KCl, and 20 mM Tris-HCl at pH 7.1 to produce a concentrated protein solution;
- (c) equilibrating a 4 µl drop of said concentrated protein solution of step (b) comprising a mixture of 1:1, v/v, acyl carrier protein synthase/reservoir solution in a 500 µl solution comprising 8-15% polyethyleneglycol 4000, 200 mM ammonium sulfate, and 100 mM citrate buffer at pH 4.5;
- (d) growing a crystal of said acyl carrier protein synthase by vapor diffusion at 294K for at least 4 to 5 days;
- (e) contacting said crystal of acyl carrier protein synthase and a solution comprising a chemical compound of interest; and
- (f) incubating said mixture to permit said compound to diffuse into said crystal.